G2 INTELLIGENCE
A DIVISION OF PLAIN LANGUAGE MEDIA

DIAGNOSTIC TESTING & Emerging Technologies

New Trends, Applications, and IVD Industry Analysis

March 2016

www.G2Intelligence.com

New Evidence Addresses Preferences for, Implications of Returning Sequencing Results

Clinical uses for comprehensive sequencing, including whole exome sequencing (WES) and whole genome sequencing (WGS) are increasing. Although some recommendations exist for the reporting of secondary findings in comprehensive sequencing, this topic remains controversial.

Concerns include psychosocial risks for patients who derive minimal or no clinical benefit from the results, as well as the implications for family members and children. Several research groups are now beginning to provide answers regarding patient preferences, as well as psychosocial and behavioral consequences of return results in large samples of patients. Recent studies show preferences for results among patients with advanced cancer, the potential benefit of returning actionable secondary results along with information on hereditary predisposition for a nonactionable condition, and the use of results among adoptees, without the context of family history.

Findings in Advanced Cancer Patients

The majority of patients with advanced cancer want both cancer-related and incidental findings from whole exome sequencing (WES), according to a study published online Feb. 11 in *Genetics in Medicine*. Patients' low levels of genetic knowledge and oncologists' inexperience with large-scale sequencing present challenges to implementing paired germline and somatic sequencing in practice, the authors say.

Continued on page 2

Multifaceted Approach Cuts Unnecessary Inpatient Lab Testing, Reduces Costs

A multifaceted intervention is able to cut inpatient lab costs per day by reducing the number of commonly ordered, but unnecessary, routine tests, according to a study published Feb. 4 in the *Journal of Hospital Medicine*. The intervention incorporates education and cost feedback to change the culture of routine test ordering into a "thoughtful process," the authors say.

Laboratory tests, while a small percent of the total health care spend, are documented to contribute to waste, with an estimated 30 percent to 50 percent of tests for hospitalized patients being unnecessary, the authors say. A

Continued on page 8

Implications of Returning Sequencing Results, *Continued from top of p.1*

Recent studies point to the increased power of sequencing results to unequivocally distinguish cancer-related genomic alterations when both somatic and germline DNA are sequenced in parallel. However, paired sequencing can also uncover previously unsuspected inherited cancer risk, which hold implications both for patient treatment, as well as their families.

"Our findings advance the field by demonstrating that although physicians anticipate many challenges to delivering care involving large-scale sequencing, patients with incurable cancer express a strong desire to learn about genomic findings whether or not they have relevance to their immediate medical care."

—Stacy Gray, M.D.

The Dana-Farber Cancer Institute's CanSeq study, launched in February 2013, evaluates the integration of paired WES in clinical care. The researchers conducted surveys and interviews to understand 27 oncologists' attitudes about WES and to identify 167, stage IV lung and colorectal cancer patients' preferences for learning WES findings.

The CanSeq researchers found that although oncologists have extensive experience ordering somatic tests (median, 100 per year), they have less experience with germline tests (median, two germline cancer predisposition tests per year). Gastrointestinal oncologists ordered or interpreted more germline tests during the prior year than thoracic oncologists, reflecting recommendations for testing for the inherited Lynch Syndrome.

Oncologists intended to disclose most WES results (both somatic and germline). Many oncologists noted that the actionability of the germline results would be a key determinant of disclosure, as they would be less willing to disclose results if it did not have implications for cancer therapy or prevention. Oncologists were also less confident in their abilities to provide psychosocial support related to negative prognostic results and cancer risk testing.

"Our findings advance the field by demonstrating that although physicians anticipate many challenges to delivering care involving large-scale sequencing, patients with incurable cancer express a strong desire to learn about genomic findings whether or not they have relevance to their immediate medical care," write the authors led by Stacy Gray, M.D. "Given the complexity of the results and the familial implications of germ-line findings, institutions that offer WES or whole genome sequencing may need to make relevant clinical and counseling expertise available to oncologists, patients, and patients' family members."

Patients had moderately low levels of genetic knowledge (mean 4 correct out of 7). Almost all patients (more than 95 percent) chose to learn most cancer-related, pharmacogenetic, and carrier status findings, but fewer chose to receive negative prognostic results (84 percent) and results suggesting predisposition to untreatable noncancer conditions (85 percent).

Findings for Dual-Risk Conditions

Disclosure of pleiotropic information may actually decrease distress among persons at increased genetic risk for two conditions, according to a study published Feb. 2 in the *Annals of Internal Medicine*. Pleiotropic genes pose a challenge to communicating genetic test results because the variant may unexpectedly confer knowledge of a separate disease risk. The ε4 allele of the apolipoprotein E (APOE) gene, which is common in the general populations and robustly associated with risk for Alzheimer disease (AD), has a weaker and less well-known association with risk for coronary artery disease (CAD).

Researchers from the Risk Evaluation and Education for Alzheimer disease (REVEAL) Study Group randomly assigned 257 participants seeking a genetic risk assessment for AD to receive risk information on AD only (AD-only) or on AD and CAD (AD+CAD). More than two-thirds of enrollees (69 percent) had an AD-affected first-degree relative. Outcomes were measured at six weeks, six months and 12 months following testing. Genetic counselors provided pre-test counseling.

The REVEAL researchers found that anxiety and depression were similar between participants receiving AD-only information and those also receiving secondary information on CAD risk. APOE ε4 carriers, those participants at increased risk for disease, experienced less test-related distress at 12 months if they also received CAD information. Participants in the AD+CAD groups also reported more health behavior changes, regardless of APOE genotype.

"Our results may help health care professionals and policy makers to better understand the desires of adopted patients and how the provision of genetic information may affect their health."

—Robert Green, M.D.

Unique Case of Adoptees

Adoptees use direct-to-consumer personal genomic testing (PGT) to gain an understanding of their genetic identities and to fill in "inaccessible information" about their family history, according to a study published online Jan. 28 in *Genetics in Medicine*. The evidence also shows that adoptees use information gleaned from PGT in a manner similar to nonadoptees.

Adoptees face a catch-22. PGT provides an easy means of obtaining personalized information regarding disease risks, inherited traits, pharmacogenomics, and ancestry. Yet, critics say giving results without the context of family history, and/or clinician interpretation, may lead to false reassurance or unnecessary alarm by results. There is currently no consensus recommendation on the appropriateness of disclosing genetic results to patients with limited family history data.

The Impact of Personal Genomics (PGen) Study surveyed new 23andMe (Mountain View, Calif.) and Pathway Genomics (San Diego) customers before and 6 months after receiving PGT results (initial recruitment from March to July 2012). Adoptees' and nonadoptees' PGT attitudes, expectations, and experiences were compared.

The PGen Study Group found that five percent of the 1,607 participants were adopted. Adoptees were ten times more likely to cite limited knowledge of family health history and 2.7 times more likely to cite the opportunity to learn genetic disease risks as strong motivations for PGT, compared to nonadoptees. In terms of PGT-motivated health-care utilization or health-behavior change, there were no significant differences between adoptees and nonadoptees. PGT results caused 41 percent of all participants to utilize a health-care service and 56 percent to change a health behavior (diet, exercise, medications, or supplements) within 6 months of receiving PGT results.

"Our results may help health care professionals and policy makers to better understand the desires of adopted patients and how the provision of genetic information may affect their health," writes senior author Robert Green, M.D., from Brigham and Women's Hospital in Boston. "It remains to be seen whether genetic-risk predictions from PGT can be useful in the absence of family history information."

Takeaway: Emerging studies are beginning to fill in uncertainty regarding the return of secondary sequencing findings among different patient populations. These findings could inform future best practices.

Non-Public Variant Reporting Hampers Consistency of Sequence Interpretation Between Labs

Discrepancies in the interpretation of variants between laboratories occurs in more than half the samples, according to a study published in the January issue of *Genetics in Medicine*. Alarmingly, many of these discrepant interpretations could significantly affect diagnosis or recommendations for clinical care. Standardization of variant interpretation could benefit from expansion of publicly available variant catalogs through mandatory reporting, standards for the use of investigative algorithms, and inclusion of predicted protein consequences in variant and clinical databases, the authors suggest.

"Of the genes interrogated in the OL inquiries, 80 percent of recorded ClinVar pathogenic entries were submitted by the CDL, which reflects a low submission rate for genes associated with heritable connective-tissue disorders to date by other laboratories."

—Melanie Pepin

The Collagen Diagnostic Laboratory (CDL; Seattle, Wash.), is a research and clinical laboratory specializing in heritable connective-tissue disorders. It commonly receives requests to provide "second opinion" on variants identified by outside laboratories (OLs). Over a 14-month period, CDL received 38 of these requests for interpretation. Interpretations by the two laboratories were compared and discrepancies were assessed. OL reports were reviewed for sources and tools used in interpretation to understand the reason for discordance between the labs.

The researchers found that the original genetic testing was completed in five private commercial laboratories (20 sample inquiries), six academic laboratories (12 sample inquiries), and six unidentified laboratories. Gene interrogation at the OL used exome sequencing (n = 6), next-generation sequencing panel (n = 17), single-gene Sanger sequencing (n = 8), or unknown methodology (n = 7). The types of variants identified included missense (n = 28), intronic (n = 6), in-frame duplications (n = 2), and synonymous (n = 1).

Interpretations between the laboratories were concordant in 11 cases (29 percent). Discrepancies were classified as moderate in 11 instances (29 percent) and significant in 16 (42 percent). In just over half (13 of 25) of cases classified by an OL as a variant of unknown significance, CDL interpreted the variant as either definitively benign or pathogenic. Factors that were identified as potential causes of the discrepancies included private data not shared in a public database (n = 9); publicly available allele frequency data not referenced and used as evidence (n = 5); and important aspects of protein structure and function that were not taken into account (n = 13).

"Of the genes interrogated in the OL inquiries, 80 percent of recorded ClinVar pathogenic entries were submitted by the CDL, which reflects a low submission rate for genes associated with heritable connective-tissue disorders to date by other laboratories," writes lead author Melanie Pepin, from University of Washington, Seattle. "With time, the increased availability of variant data should decrease discrepant interpretations among laboratories that result because data are available to only one source. However, submission of all accrued variants from prior years and decades of testing faces the logistical challenge of insufficient laboratory resources to allocate to the task of manual curation and submission."

Takeaway: Discrepancies between laboratories in the interpretation of variants are common and can affect clinical care. Lack of data sharing of variant information is responsible for many of these interpretation discrepancies. G2

Inside The Diagnostics Industry

Proteomics, Metabolomics Could Stratify Women at Risk for Common, But Serious Pregnancy Conditions

Noninvasive prenatal testing (NIPT) for trisomies has dominated the obstetrics-related diagnostic headlines and masked the lack of development for diagnostics for other pregnancy-related conditions. While rapid adoption of NIPT is improving clinical obstetric care, clinicians see a desperate need for diagnostics development in the underserved areas of common, but adverse, conditions of pregnancy like preterm birth and preeclampsia.

Experts say that costs associated with preterm birth are on average more than $54,000, or ten times higher than the cost of a baby delivered at full term.

"NIPT has been driven by technological advancements. Next-generation sequencing has made it easier to get access to genetic information," Robin Tuytten, Ph.D., vice president research and development at Metabolomic Diagnostics (Ireland) tells *DTET*. "We can get our heads around it because screening for chromosomal aberrations builds off of existing tests in the market place."

Louise Kenny, M.B.Ch.B., Ph.D., from Cork University Maternity Hospital in Ireland says that there has been nearly no new development of obstetrics screening tests, besides NIPT, in nearly 30 years. She acknowledges conditions like preeclampsia and preterm birth are complex syndromes, but have been neglected in terms of research and development by both public and private entities.

Preterm Birth

Experts say that costs associated with preterm birth are on average more than $54,000, or ten times higher than the cost of a baby delivered at full term. Preterm birth is common, with the U.S. Centers for Disease Control and Prevention saying that one of every 10 births is affected. Preterm birth is tied to neonatal and early childhood morbidity as well as increased risk of major long-term medical complications, including learning disabilities, cerebral palsy, and vision and hearing loss.

Frustratingly to care providers, the majority of preterm births are spontaneous and not tied to known prenatal or maternal medical conditions. Prior history of spontaneous preterm delivery and cervical length measurements are considered the best measures of clinical risk to date, but individually or in combination, they fail to predict the majority of spontaneous preterm deliveries. Researchers are hopeful that emerging diagnostics will soon be able to identify women at higher risk of preterm birth through biomarkers early in pregnancy, before symptoms appear.

"The intrauterine space is both physically and ethically remote. As such, this is perhaps why, with the possible exception of the measurement of cervical length by ultrasound, little recent progress has been made in the development of useful biomarkers to stratify patients according to risk of SPTB," writes David Cantonwine, Ph.D., from Brigham and Women's Hospital in Boston, Mass., in a study published Feb. 10 in the *American Journal of Obstetrics & Gynecology* (*AJOG*). "The evolving field of circulating microparticle biology may offer a solution to these difficulties as these particles present a sampling of the utero-placental environment. Additionally, studying the contents of these particles holds the promise of identifying novel blood-based, and possibly clinically useful, biomarkers."

Inside The Diagnostics Industry

In the *AJOG* study, the researchers found functional proteomic factors, known to be associated with biological processes that already had distinguishable expression profiles at 10 to 12 weeks gestational age among women who go on to deliver spontaneously at 34 weeks or less. The researchers analyzed plasma specimens obtained between 10 and 12 weeks gestation as part of a prospective birth cohort. They matched 25 singleton cases of spontaneous preterm birth at 34 weeks or less and 50 uncomplicated term deliveries using factors such as maternal age, race, and gestational age of sampling. Circulating microparticles from these specimens were isolated and analyzed with quantitative proteomic liquid chromatography-mass spectrometry to identify potential protein biomarkers.

"Differences in the protein content of microparticles likely represent an untapped source of information regarding biology of the maternal-fetal interface."

—David Cantonwine, Ph.D.

The researchers found that 62 of 132 identified proteins demonstrated robust power of detecting spontaneous preterm birth. These proteins were tied to biological processes of inflammation, wound healing, and the coagulation cascade. Using a multiplex of the candidate biomarkers had a sensitivity of 80 percent and a specificity of 83 percent with median area under the curve of 0.89 to predict spontaneous preterm birth, showing "strong potential" for informative risk stratification, the authors say.

"Differences in the protein content of microparticles likely represent an untapped source of information regarding biology of the maternal-fetal interface," write the authors, two of whom report financial ties to the diagnostics company NX Prenatal (Louisville, Ky.), which funded the study. "Some may suggest that we are premature in attempting the development of a predictive test without a universally agreed upon therapeutic modality… Such identification would allow the application of increased observation and the possible application of prophylactic therapies such as progesterone, which together may significantly improve the management of these patients."

Another company working towards the development of diagnostics capable of identifying personal risk of delivering early is Sera Prognostics (Salt Lake City). The company's PreTRM blood test is performed as early as 19 weeks and measures proteins associated with preterm birth. The proteins cover pathways associated with inflammation, hemorrhage, stress, uterine over-distention. PreTRM test is performed using liquid chromatography-tandem mass spectrometry. Researchers recently reported on the test's performance in an article published by *AJOG* and at the Pregnancy Meeting—the annual meeting of the Society for Maternal Fetal Medicine (Feb. 1-6; Atlanta, Ga.).

The 11-site PAPR (Proteomic Assessment of Preterm Risk) study enrolled 5,501 pregnant women (gestational age, 17 to 28 weeks between 2011 and 2013), representative of the United States population. Based on the researchers' previous proteomic findings, they validated a signature based on two proteins that are highly predictive of preterm birth risk: IBP4, insulin-like growth factor binding protein 4, and SHBG, sex-hormone binding globulin. The predictor (the ratio of IBP4/SHBG levels at 19 to 20 weeks gestation) had an area under the receiver operating characteristic curve value of 0.75 and sensitivity and specificity of 0.75 and 0.74, respectively.

Inside The Diagnostics Industry

"The classifier performance of the proteins in the PAPR study was excellent," said George R. Saade, M.D., from the University of Texas Medical Branch in Galveston and lead investigator. "These data demonstrate the powerful role proteomics can play in giving physicians a new tool to predict early an individual woman's risk of spontaneous preterm birth."

"As preeclampsia cannot be predicted by previous obstetric history and risk factors alone, much research has focused on the identification of women at high risk of developing preeclampsia."

—Louise Kenny, M.B.Ch.B., Ph.D.

Preeclampsia

Preeclampsia is one of the most common complications during pregnancy, occurring in about one in every 20 third-trimester pregnancies. Similar to spontaneous preterm birth, there are currently no reliable biomarker tests for preeclampsia that have been accepted for wide clinical use, according to a review published in the fall of 2015 in the *International Journal of Molecular Science*.

"As preeclampsia cannot be predicted by previous obstetric history and risk factors alone, much research has focused on the identification of women at high risk of developing preeclampsia," writes co-author Louise Kenny. "This would allow more intensive monitoring of this high-risk group as well as targeted prophylactic intervention, timely diagnosis and treatment."

The researchers identified 147 studies that described 401 laboratory biomarkers. Placental growth factor (PlGF), pregnancy associated plasma protein A (PAPP-A), soluble fms-like tyrosine kinase (sFLT), and placental protein 13 (PP-13) were the most commonly studied biomarkers. The review found low predictive values for several individual biomarkers including a disintegrin and metalloprotease 12 (ADAM-12), inhibin-A, PAPP-A, PIGF, and PP-13. In pooled analysis, the sensitivity of all single biomarkers was 0.40 with a false positive rate of 10 percent. The area under the summary of receiver operating characteristics curve was 0.786. The authors concluded that, "although there are multiple potential biomarkers for preeclampsia, their efficacy has been inconsistent and comparisons are difficult because of heterogeneity between different studies."

Kenny's research has been licensed by Metabolomic Diagnostics (Ireland), which is currently conducting clinical trials to validate a preeclampsia test and has a test for preterm birth also in the pipeline. Early research showed promising results for the preeclampsia test, which is based on a unique set of 14 metabolites that can be identified from a blood sample taken at 15 weeks of gestation. The early evidence is based on samples from the Screening for Pregnancy Endpoints (SCOPE) study and bio-bank, which includes multiple samples from 6,000 participants in six countries. Next steps include further validation of this multi-metabolite based test in the large scale European, multicentre phase IIa clinical study IMproved PRegnancy Outcome by Early Detection (IMPROvED) which is currently recruiting first time pregnant women in five European countries.

Takeaway: Proteomics and metabolomics are enabling a new renaissance in the field of obstetrics. There is hope that new screening tests for spontaneous preterm birth and preeclampsia will reverse decades of stagnation in the pursuit of identifying women at early risk for these conditions.

Multifaceted Approach Cuts Unnecessary Inpatient Lab Testing, *Continued from bottom of p.1*

University Health Systems Consortium 2011 analysis indicated that The University of Utah General internal medicine service compared their costs to 2011 University Health Systems Consortium's data and determined it had a higher average direct lab cost per discharge compared to top performers. In addition to the potential cost savings, potential anemia from phlebotomy during long hospital stays, negative patient experience from frequent, early morning blood draws, and a cascade effect from false positives that lead to further testing and monitoring all contributed to the hospital's interest in quality improvement efforts.

> *"Prior to this intervention, the least experienced person on this team, the intern, ordered any test he or she wanted, usually without discussion."*
>
> —Peter Yarbrough, M.D.

University of Utah Health Care developed a Value Driven Outcomes (VDO) tool to give direct data related to costs of care, including the actual cost paid by the hospital to the university-owned laboratory vendor (ARUP Laboratories) for testing. The VDO provided routine cost feedback. Additionally, the intervention included education for all hospitalist group providers on laboratory overuse and costs and standardization of the rounding process to include a checklist review that ensured discussion of lab testing. Lastly, a shared savings program would provide 50 percent of realized cost savings back to internal medicine to support future quality improvement projects, but physicians did not personally benefit financially.

The baseline period (July 1, 2012 to Jan. 31, 2013) was compared to the intervention period (Feb. 1, 2013 to April 30, 2014). This study included 6,310 hospitalist patient visits (internal medicine; intervention arm) and 25,586 non-hospitalist services (surgical services, pulmonary, cardiology, hematology, and oncology services; control arm).

The researchers found that in the intervention group, unadjusted mean cost per day was significantly reduced from $138 before the intervention to $123 after the intervention and unadjusted mean cost per visit decreased significantly from $618 to $558. The number of tests per day significantly decreased for all specific tests (basal and comprehensive metabolic panels and complete blood chemistry tests) in the intervention group. The authors projected that the decreased cost in the intervention group amounts to approximately $251,427 savings over the first year and could have led to an additional cost savings of $1,321,669 if the intervention was used and had similar impact in the control group. In addition to the cost savings, readmission rates decreased significantly by 3 percent in the intervention group, but length of stay was unchanged.

"Prior to this intervention, the least experienced person on this team, the intern, ordered any test he or she wanted, usually without discussion," write the authors led by Peter Yarbrough, M.D., University of Utah, Salt Lake City. "The intervention focused on this issue through standardized supervision and explicit discussion of laboratory tests. … The incorporation of process change in this intervention was felt to likely contribute to the sustained reduction seen at 15 months."

Takeaway: A multifaceted intervention targeting unnecessary inpatient hospital testing could save $1.5 million a year in an academic medical center setting. G2

Annual Self-Screening With FIT Effective for Colorectal Cancer Detection

Annual, programmatic fecal immunochemical test (FIT) screening for colorectal cancer (CRC) is both feasible and effective in a large community-based setting, according to a study published Jan. 26 in the *Annals of Internal Medicine*. Annual FIT screening has high sensitivity for CRC and maintains high adherence to annual follow-up screening among initial participants.

Annual, high sensitivity fecal occult blood tests are believed to be as effective as colonoscopy screening every 10 years, if levels of adherence are high, the authors say. In addition to being noninvasive and deliverable by mail, FIT screening can be performed without dietary or medication restrictions, giving it higher patient acceptance. Despite its promise to boost CRC screening adherence, most research to date, the authors say, evaluated FIT test performance in the first round, not how it does in later rounds of repeat testing.

FIT Follow-Up Varies

There is significant variation in the time between patient's receipt of positive fecal blood test results and the follow-up colonoscopies, according to a study published Feb. 4 in *Cancer Epidemiology, Biomarkers and Prevention*. While health care system factors influence the length of this lag, laboratories may able to help improve processes involving return of abnormal test results.

Using data from the Population-Based Research Optimizing Screening through Personalized Regimens (PROSPR) consortium, researchers identified 62,384 individuals (aged 50 to 89 years) with a positive fecal blood test (fecal occult blood tests or fecal immunochemical tests) between 2011 and 2012 in four U.S. health care systems.

The researchers found that most patients who received a colonoscopy did so within 6 months of their positive fecal blood test result, although follow-up rates varied significantly across health care systems (median range, 41 to 174 days, while the percent followed-up by 12 months ranged from 58.1 percent to 83.8 percent). Increasing age and comorbidities were associated with lower follow-up rates, but health system differences persisted at 1, 2, 3, and 6 months.

"Data support the importance of organizational factors in the completion of diagnostic work-up of positive fecal blood tests," write the authors led by Jessica Chubak, from the Group Health Research Institute in Seattle, Wash. "There is increasing interest in studying follow-up to abnormal screening tests in a multilevel context…. Such studies are needed to lay the groundwork for future research on improving the effectiveness of cancer screening."

In the present study, researchers identified 323,349 Kaiser Permanente Northern California and Southern California health plan members (aged 50 to 70 years) on the FIT mailing list (in 2007 or 2008), who completed the first round of FIT. Patients were followed for up to 4 screening rounds.

The researchers found that of the patients invited for screening, 48.2 percent participated in the first round. Of those who remained eligible, 75.3 percent to 86.1 percent of round one participants also took part in subsequent rounds. Across all screening rounds, 63.8 percent of distributed tests were completed within one year of mailing. Over the four screening rounds, 7 percent of patients crossed over to endoscopy because of a positive FIT test.

Round one had the highest FIT positivity rate (5.0 percent) and positive predictive values (adenoma, 51.5 percent; CRC, 3.4 percent). These lower, but relatively stable measures in subsequent rounds, likely reflect prevalent cancers initially and incident cancers subsequently. Following this same pattern of prevalence cancer, the FIT sensitivity for CRC was highest (84.5 percent) in the first screening round and declined to a more steady state (range in sensitivity, 73.4 percent to 78.0 percent) in subsequent rounds of screening.

Takeaway: Patients who initiate FIT screening for CRC are highly likely to adhere to an annual screening schedule. Furthermore, repeated FIT testing has a high sensitivity to detect CRC in a large community cohort. G2

Inflammatory Markers May Diagnose Back Conditions

Biochemical profiles of blood may improve diagnosis of conditions contributing to low back pain (LBP), according to a study published online Jan. 7 in *Arthritis Research & Therapy*. LBP is associated with low-grade systemic inflammation and circulating cytokine levels may differentiate disc-related causes of pain.

LBP is a widespread and costly problem. It is the second most common cause of physician visits in the United States, affects the majority of people at some point during their lifetime, and is estimated to cost $50 billion to $100 billion in direct health care spending. Yet, timely and definitive diagnosis is a challenge as pain can have multiple potential causes that present similarly and respond unpredictably to treatment. Additionally, MRI imaging has been shown to have poor correlation to functional impairment or pain.

The researchers recruited participants (average age 50 years; 43 percent male) with LBP from a spine neurosurgery practice (n=80) and a back pain management practice (n=27), as well as a control cohort (n=26). Serum samples were collected before treatment (either epidural steroid injection or surgery) and were assayed by multiplex electrochemiluminescence immunoassays for levels of multiple interleukins (IL), interferon-γ, tumor necrosis factor-α, and multiple matrix metalloproteinases (MMPs).

Controlling for age and gender effects, participants with LBP (combined epidural and surgery cohorts) had serum IL-6 levels that were significantly (41 percent) higher than control participants. Participants with LBP due to spinal stenosis or degenerative disc disease had higher IL-6 levels than those with intervertebral disc herniation and controls. In contrast, serum levels of MMP-1 were significantly (56 percent) lower in LBP participants than in controls. MMP-1 levels were lower specifically in patients with disc herniation versus control subjects. There were no significant differences in IL-6 or MMP-1 serum levels with respect to disease severity (classified by Pfirrmann grade).

"It is unknown if circulating cytokines are causative of degenerative changes and pain or whether elevated cytokine levels are a consequence of the degeneration and painful condition," write the authors led by Kathryn Weber, M.D., a clinical research fellow from the North Shore-LIJ Health System. "The possibility of using serum cytokine measures of IL-6 to predict response to treatment with progressively targeted therapies fits the emerging concept of personalized molecular medicine, where treatments are tailored to an individual's specific biology through increasingly sensitive detection of molecular abnormalities, and the use of these inflammatory mediators as biomarkers for LBP."

Takeaway: Diagnosis and treatment of LBP caused by disc disease may be improved through the use of biochemical profiling of circulating cytokines.

NGS-Based Test for CF Promises Faster, Cheaper, More Thorough Analysis

Testing for the cystic fibrosis (CF) helped to fuel the first wave of clinical molecular diagnostics. Now, an assay developed by researchers at the Stanford University taps the power of multiplex next-generation sequencing (NGS) to enable early detection and management of CF in ways that could consolidate sample types and technologies.

The new assay, known as CFseq, reliably sequences the entire CFTR gene from a single 3.2-millimeter newborn DBS punch sample. This is the first time scientists have found a way to reliably use DBSs for this type of sequencing for CF, which typically requires much more DNA. Moreover, the one-step method costs less and takes about half the time of current testing methods.

The highly sensitive, specific, rapid, and potentially cost-effective new test is described in a paper published in the March issue of the *Journal of Molecular Diagnostics*.

Cystic fibrosis is the most common fatal genetic disease in the United States. Newborns in every U.S. state have been screened for CF since 2010, but the current tests vary widely and have limitations.

"The assays in use are time-consuming and don't test the entire cystic fibrosis gene," says the study's senior author, Curt Scharfe, M.D., Ph.D., now at Yale University. "They don't tell the whole story."

Most CF tests now in use begin with immunoreactive trypsinogen testing of dried blood spots (DBSs) taken from newborn heel sticks. Since this assay is sensitive but prone to false positives, newborn screening programs rely on tiered strategies, which reflex hypertrypsinogenemic specimens to methods that interrogate a relatively small number of common CF mutations. If one of the common mutations is identified, the infant's entire CF gene is sequenced to try to confirm whether the baby has a second, less common CF mutation. The process takes up to two weeks and can miss infants who carry two rare CF mutations, particularly in ethnically diverse populations.

The new assay, known as CFseq, reliably sequences the entire CFTR gene from a single 3.2-millimeter newborn DBS punch sample. This is the first time scientists have found a way to reliably use DBSs for this type of sequencing for CF, which typically requires much more DNA. Moreover, the one-step method costs less and takes about half the time of current testing methods.

"In our new assay, we are reading every letter in the book of the CF gene," says study co-author Iris Schrijver, M.D., from Stanford. "Whatever mutations pop up, the technique should be able to identify. It's a very flexible approach."

In addition to NBS, the test could be used for carrier and diagnostic testing and broadened to screen for other genetic diseases, not just CF.

Stanford's Molecular Pathology Laboratory has a contract with California for the state's CF newborn testing, so the immediate next steps are staff training and clinical validation of the new assay. California newborn screening officials will then have the opportunity to decide whether they want the new test to replace the current method. Schrijver expects the process will take less than a year.

Takeaway: NGS using DBS provides a more thorough, time efficient, and less costly way to screen newborns for CF. G2

G2 INSIDER

Acute HIV Screening With Combo Assay Ups Diagnostic Yield

Use of an HIV antigen/antibody (Ag/Ab) combination assay increases the diagnostic yield by more than 10 percent compared with rapid HIV testing in a high-prevalence population, while pooled RNA testing further increases the diagnostic yield, according to a study published in the Feb. 16 issue of the *Journal of the American Medical Association*. Because rapid testing detected HIV infection in only 87 percent of HIV-infected participants, the authors say alternative strategies that can detect acute infection should be considered in high-prevalence, U.S. populations.

Acute HIV infection is defined as the interval between the appearance of HIV RNA and detection of HIV-specific antibodies and can be diagnosed with HIV RNA assays (the reference standard) or the p24 antigen. The U.S. Centers for Disease Control and Prevention (CDC) recommends an HIV diagnostic algorithm that uses HIV immunoassays that detect both the p24 antigen and anti-HIV antibody (fourth generation Ag/Ab combo assay) as the initial screening test. But, they are not as sensitive as pooled HIV RNA tests, which are not widely used because there is only one U.S. Food and Drug Administration-approved assay and the protocol is complex.

This prospective trial (September 2011 through October 2013) included seven sexually transmitted infection clinics and five community-based programs in New York, California, and North Carolina. All participants (median age, 29 years; seeking HIV testing, without previously known infection) with a negative rapid HIV test result were screened for acute HIV infection with an HIV Ag/Ab assay (index test) and a pooled HIV-1 RNA test (reference standard).

The researchers found that among 86,836 participants with complete test results, established HIV infection was diagnosed in 1.33 percent of participants and acute HIV infection was diagnosed in 0.19 percent. HIV Ag/Ab combination testing detected acute HIV infection in 134 of 168 participants (sensitivity, 79.8 percent; specificity, 99.9 percent; positive predictive value, 59.0 percent) versus HIV RNA testing which detected 164 of 168 participants (sensitivity, 97.6 percent; specificity, 100 percent; positive predictive value, 96.5 percent). HIV Ag/Ab testing detected 82 percent of acute HIV infections detectable by pooled HIV RNA testing. Compared with rapid HIV testing alone, HIV Ag/Ab combo testing increased the relative HIV diagnostic yield by 10.4 percent, while pooled HIV RNA testing increased the relative HIV diagnostic yield by 12.4 percent.

The authors, led by Philip Peters, M.D., from the CDC, suggest two scenarios for incorporating Ag/Ab combo testing into screening regimens. In one scenario, for patients with adequate contact information, laboratory-based Ag/Ab combination testing could replace the rapid HIV test for initial screening. In the second scenario, if rapid testing is negative, high-risk individuals could be offered an additional laboratory-based Ag/Ab testing to diagnose acute HIV infection potentially missed with rapid testing, although this approach would be unsuited to low–HIV prevalence populations due to the test's low positive predictive value. G2

Company References

Collagen Diagnostic Laboratory
206-543-0459

Kaiser Permanente Northern California
510-891-3400

Metabolomic Diagnostics
+353 (0)21 7011171

NX Prenatal
502-893-4570

Sera Prognostics
801-990-0520

Stanford University Molecular Pathology Laboratory
650-723-6574

University of Utah
801-581-7606

U.S. Centers for Disease Control and Prevention
800-232-4636

 Diagnostic Testing and Emerging Technologies (ISSN 2330-5177) is published by G2 Intelligence, Plain Language Media, LLLP, 15 Shaw Street, New London, CT, 06320. Phone: 1-888-729-2315 • Fax: 1-855-649-1623. Web site: www.G2Intelligence.com.

Kelly A. Briganti, JD, Editorial Director, Kelly@plainlanguagemedia.com; Lori Solomon, Editor; Barbara Manning Grimm, Managing Editor; Stephanie Murg, Managing Director; Kim Punter, Director of Conferences & Events; Randy Cochran, Corporate Licensing Manager; Jim Pearmain, General Manager, Pete Stowe, Managing Partner; Mark T. Ziebarth, Publisher.

Receiving duplicate issues? Have a billing question? Need to have your renewal dates coordinated? We'd be glad to help you. Call customer service at 1-888-729-2315.